INVESTIGATIONS OF THE PREFERENCES SHOWN BY *AEDES* (*STEGOMYIA*) *AEGYPTI* LINN. AND *CULEX* (*CULEX*) *FATIGANS* WIED. FOR SPECIFIC TYPES OF BREEDING WATER.

By Tom Manefield, B.Sc.,

Department of Zoology, University of Sydney.

[Read 29th August, 1951.]

Synopsis.

Comparative experiments are outlined showing the reaction of *Aëdes aegypti* and *Culex fatigans* to two types of water, representative of field habitats, these being tap water and a "foul" medium for which a manure infusion was used. Results are given showing the oviposition responses of the adult females to both types of water and the survival percentages of the larvae of the two species when reared in these media.

Each species showed preference for the foul medium for oviposition, that shown by *C. fatigans* being complete. *C. fatigans* larvae were more able to survive than *A. aegypti* in the foul medium at all stages of introduction into infusions of varying maturities. It was also shown that development of a scum on the manure infusions was more lethal to the latter species.

Introduction.

The reason for the differences shown by mosquitoes in the selection of larval habitats is a problem which has received much attention from various workers. From early observations in the field it was obvious that the occurrence of mosquito larvae was not haphazard and that no one species is found in all types of water, marked preferences being noticeable. Classifications of habitat have been given by Hopkins (1936), who proposed a grouping according to the situation of the water (e.g. ground water, rock pools), and Lee (1944) whose classification is based on the state of the water itself (e.g. fresh, polluted). During earlier investigations masses of physico-chemical data were obtained by such workers as MacGregor (1929), Senior-White (1934), Beattie (1932). Few significant results were obtained, but Buxton (1934), examining Beattie's results, shows that her conclusion correlating larval prevalence with ammonia nitrogen was significant. More recently work by Thompson (1940–41), Woodhill (1941) and others concerning the selection by the adult female of the site of oviposition seems to be of a more positive nature.

In this investigation *A. aegypti* and *C. fatigans* were selected as representing typical cases of different breeding habits. *A. aegypti* is an entirely domesticated species, breeding in and around human dwellings, usually in fresh water contained in artificial receptacles. It is rarely recorded in foul water, whereas *C. fatigans*, on the other hand, shows a distinct preference for such water. This investigation is of a preliminary nature and was approached from two angles, namely, oviposition responses, comparing the reaction of the two species to water which was believed to be typical of the location in which the larvae have been recorded; and larval experimentation, to see if there was any effect shown by the rearing of the species found in one habitat type, in the alternate type. The two media used were a horse manure infusion and tap water (i.e. the normal reticulated water supply of Sydney, which contains 0·0062% dissolved salts).

Breeding Technique.

The *A. aegypti* eggs were obtained from a stock which has been continuously bred at the Zoology Department, University of Sydney, since 1938. Eggs of *C. fatigans*, which were collected from tubs outside the laboratory, were usually obtainable in sufficient numbers all the year round, but in winter it was at times necessary to maintain a laboratory culture. The technique used for breeding mosquitoes was similar to that used by Woodhill (1936). A laboratory culture of *A. aegypti* was continuously maintained, as fresh eggs were used during the larval experimentation.

This was also done with *C. fatigans* when sufficient eggs were not obtainable outside the laboratory.

The "foul" medium was prepared by mixing approximately equal volumes of dried horse manure and water. These infusions were allowed to mature for three different periods—7 days, 10–11 days, and 14–15 days. They were made up at varying times so that the three states of maturity required would be available at the same time.

Experimentation.

Oviposition Responses.

Males and females of the two species (about 100 of each) were released into separate uniform cages, 12″ × 10″ × 9″, and given blood feeds and raisins. In each cage

Table 1.
Number of Eggs Deposited by C. fatigans *and* A. aegypti.

Observation Number.	*Culex fatigans.*				*Aëdes aegypti.*	
	Tap Water.		Manure Infusion.		Tap Water.	Manure Infusion.
	Number of Rafts.	Number of Eggs.	Number of Rafts.	Number of Eggs.	Number of Eggs.	Number of Eggs.
1	Nil	Nil	4	299	299	489
2	,,	,,	7	455	440	727
3	,,	,,	9	571	560	960
4	,,	,,	9	612	138	436
5	,,	,,	1	75	130	613
6	,,	,,	9	635	89	741
7	,,	,,	7	483	548	2,765
8	,,	,,	10	780	75	180
9	,,	,,	5	381	1,009	1,841
10	,,	,,	5	351	545	1,521
11	,,	,,	21	1,552	77	883
12	,,	,,	9	678	105	747
13	,,	,,	3	156	195	4,990
14					11	153
15					166	805
16					495	3,472
Mean percentages ..		0		100	17·7	82·3

were placed two oviposition dishes, one containing tap water and the other "foul" water which had stood for at least ten days before use. They were placed at opposite ends of the cage, the position reversed daily, and the cages placed parallel to the window in order to eliminate any positional or phototactic response. The eggs were collected and counted at regular intervals, and a record kept of the data and numbers of females feeding. After collection of the eggs the contents of the oviposition dishes were always replaced. This procedure was followed even when no eggs had been laid. Results are given in Table 1.

Under statistical analysis the preference shown by both species for foul water proved significant, giving the probability $P = \frac{1}{2^{32}}$.

Experiments with Larvae.

Fifty larvae at known stages were placed in uniform dishes containing 200 c.c. of the manure infusions (at the required stages of maturity) plus food and incubated at 80°F. In each experiment controls were kept by placing larvae in tap water with food in the normal manner. The amount of food used in each case was approximately 0·35 gm. The cultures were incubated and the pupae removed as they developed until all the surviving larvae had pupated. The numbers of mature pupae resulting were recorded.

The possibility of interference by scum formation was taken into account. For comparison an effort was made in some cases to find the effect of the water alone, without the interference of the scum. Removal of the scum daily with cotton wool was satisfactory.

TABLE 2.

Percentage Aëdes aegypti *Surviving to Mature Pupal Stage.*

Larvae Stage of Introduction.	Scum Condition on Surface.	Number of Replications.	Percentage Survival.			
			Control. In Tap Water.	In Manure Infusions.		
				7 Days Maturing.	10 Days Maturing.	14 Days Maturing.
50 3rd stage larvae in each replication	Present.	6	99	70	88	98
50 2nd stage larvae in each replication	Present.	6	99	38	74	98
50 2nd stage larvae in each replication	Removed.	9	96	44	80	
50 1st stage larvae in each replication	Present.	6	96	0	58	94
50 1st stage larvae in each replication	Removed.	9	96	3	82	
50 eggs introduced into each replication	Present.	12	99	4	44	94
50 eggs introduced into each replication	Removed.	6	99	9	90	

Table 2 gives the results obtained for *A. aegypti* at known stages of introduction into the "foul" medium cultures. These figures are compared with the controls taken through at the same time in tap water.

Obviously from the results the lethal stage is during the first larval stage, probably when newly emerged, so this stage was selected for a comparison of the effect on the two species. Table 3 gives the results obtained when 50 newly emerged first-stage larvae were introduced into each culture.

TABLE 3.

Percentage of A. aegypti *and* C. fatigans *Surviving to Mature Pupal Stage.*

Scum Condition on Surface.	Number of Replications.	*Aëdes aegypti.*			*Culex fatigans.*		
		In Tap Water. Control.	In Manure Infusion.		In Tap Water. Control.	In Manure Infusion.	
			7-Day Infusion.	11-Day Infusion.		7-Day Infusion.	11-Day Infusion.
Present ..	6	99%	3%	60%	99%	62%	92%
Removed ..	6	99%	5%	84%	99%	74%	96%

The figures in Table 2 and Table 3 show the *percentage survival* of the two species. For use in the summary these figures have been converted to *percentage mortality* by subtraction from the 100% level.

Discussion.

Oviposition Responses.

The potentialities of any water as a breeding ground depend primarily upon the response of the female to that water. With the possibilities of phototactic and positionary responses eliminated, results for *C. fatigans* apparently ratified field observations. This species showed a complete preference for foul water, laying 100% of the eggs on this medium. In the field, *C. fatigans* is generally recorded in water with a high organic content. The occurrence of this species in clean water would be due to the inability of the female to find a more suitable breeding place.

A. aegypti did not show a complete preference for the "foul" water, but nevertheless did have a very marked preference. In a total of 16 observations, when 29,905 eggs were laid, 82% were deposited on the "foul" water. In the field, even when foul water of the type used is available, *A. aegypti* larvae have rarely been recorded in it and almost invariably occur in the water contained in rain water tanks and similar containers. In all its usual breeding places the organic content is relatively low, yet in the laboratory, under optimum conditions, the species preferred to lay its eggs on water with a high organic content. These results seem to correspond with those obtained by Dunn (1927). He found that, by placing containers with various types of water in the open and allowing *A. aegypti* to breed at will, the species showed a distinct preference for water to which leaves had been added, rather than ordinary tap water. Apparently *A. aegypti* is attracted by some factors which are indicative of a higher food content.

From the evidence of these findings further oviposition experimentation is indicated, giving a comparison of varying degrees of foulness or putrefaction rather than a comparison with tap water, and also types of putrefaction, for example, using leaves. It must also be noted that during these experiments the infusions used were all over ten days' maturity. Considering the larval findings with infusions of seven days' maturity, it may be interesting to see the results of similar experiments using only the seven-day infusion. A rejection point may be obtainable, giving a lead to the actual limiting factor.

Larval Cultures.

The horse manure infusion proved a satisfactory medium and was easy to handle. Initial observations were made on these infusions by exposing them to field conditions so that the approximate time of entry of *C. fatigans* could be determined, giving the time at which the infusion became a potential breeding ground. As the seventh day after preparation was found to be the critical time for laying of eggs, it was decided to see if there was any effect on *A. aegypti* by placing larvae in this medium. As *C. fatigans* was then able to breed in these cultures it was considered unnecessary to carry out comparable experiments between these species at first. The method was altered variously to show the effect of infusions of different degrees of maturity on the larval stages. Table 2 shows the results in a condensed form.

No statistical analysis was necessary for the verification of these results. The major diminution of the population, such as in the introduction of first-stage larvae and eggs into the seven-day infusion, was obviously significant. The effect of the seven-day infusions on all stages was the most noticeable. The earlier the larval stages at introduction, the greater the mortality became; however, there was a slight rise in survival with the introduction of eggs. In all cases the removal of the scum led to a slight rise in survival rate. After ten days' maturity the infusions became much less toxic, but still showed this increase in mortality with the introduction of earlier larval stages. The scum exerted a similar effect in these infusions, and with its removal the percentage survival increased appreciably. This effect was greatest on first stage larvae eggs. The 14-days infusion exerted no major influence on survival, for in all cases it was almost normal. Scum formation in these experiments was only slight when present, and it was never considered necessary to remove it.

Having shown that the manure infusions had a definite toxic effect on the larvae of *A. aegypti*, experiments were then carried out to compare the results with those obtained from *C. fatigans*. Due to the difficulty encountered in setting up exact numbers of *C. fatigans* eggs because of the raft form, it was decided to use newly hatched first-stage larvae. This stage also presented the most critical stage in the development of *A. aegypti* in the infusions. The results are shown in Table 3. For the seven-day infusions the percentage survival of *A. aegypti* was 3%, compared with 62% for *C. fatigans*. These increased to 5% and 74% when the scum was removed. For the eleven-day period the percentages were: *A. aegypti* 60%, and *C. fatigans* 92%, with the scum left, and increasing to 84% and 96% when the scum was removed. The results here for *A. aegypti* also closely correspond with those previously shown in Table 2.

A limiting factor is only evident for the seven-day infusions. As well as preferring the manure infusion for egg laying, *A. aegypti* showed that they were quite able to breed after the infusion was 14 days matured. At seven days the infusions were almost completely toxic and could prevent breeding. At 10–11 days the larvae were able to survive in sufficient numbers to set up a large community. With this increasing ability to survive in the maturing medium the lethal factor is lost. At this time some other factor must come into existence in the field which deters the setting up of a community. As well as showing a complete preference for oviposition, *C. fatigans* showed a greater ability to survive in the manure infusion. The scum forming on the cultures was shown to have more effect on *A. aegypti* than on *C. fatigans*. This is probably due to the form of the respiratory siphon. The scum, however, was not the sole deterrent factor on *A. aegypti*. It is regretted that a method for measuring the scum satisfactorily was not devised. In view of the work carried out by Beattie (1932), it is suggested that the lethal factor present is related to a change in the concentration of some nitrogen product.

Summary.

1. *Aëdes aegypti*, when allowed a choice between clean tap water and foul water (manure infusion) for oviposition, showed a distinct preference for foul water, the percentages of eggs deposited on the two types of water being 17·7% and 82·3% respectively.

2. *Culex fatigans*, when allowed a choice, showed complete preference for the foul water, no eggs whatever being deposited on tap water.

3. In the seven-day manure infusions *A. aegypti* larvae showed a higher mortality the younger the stage exposed. The percentage mortality in each stage was: larvae introduced at the third stage gave 30% mortality; second stage, 62%; first stage, 98½%; eggs, 96%.

4. A similar result was obtained with *A. aegypti* in 10–11 day manure infusions; but, even with early stages, there was sufficient survival to allow successful breeding. The mortality percentages were: third-stage larvae introduced, 12%; second-stage larvae, 26%; first-stage larvae, 41%; eggs, 56%.

5. *A. aegypti* larvae developed almost as well in 14–15 day infusions as in the controls.

6. Scum formation was shown to have some effect on survival of *A. aegypti* in the 7-day and 10–11 day infusions. The percentage mortality decreased in all cases with the removal of the scum. The effect of the scum was most noticeable with the introduction of eggs and first-stage larvae in 10–11 day infusions, the percentage mortality decreasing to 10% and 17% respectively when the scum was removed. (Cf. par. 4.)

7. *C. fatigans* was more able to survive in foul water in all experiments than *A. aegypti*. The actual mortality percentages with first-stage larval introduction were as follows:

(*a*) In seven-day infusions with the scum untouched, *A. aegypti* showed 97% mortality and *C. fatigans* 38%.

(*b*) With the scum removed these decreased to 95% and 26% respectively.

(*c*) In 11-day infusions with the scum untouched, *A. aegypti* showed 40% mortality and *C. fatigans* 8%.

(*d*) With the scum removed these decreased to 16% and 4% respectively.

ACKNOWLEDGEMENTS.

The author's thanks are due to Dr. A. R. Woodhill for his advice while carrying out this work, and to him, Professor P. D. F. Murray, and Dr. W. A. McDougall, Queensland Department of Agriculture and Stock, for many helpful criticisms during the preparation of this paper.

References.

BEATTIE, M. V. F., 1932.—The physico-chemical factors of water in relation to mosquito breeding in Trinidad. *Bull. Ent. Res.*, 23 : 477.

BUXTON, P. A., 1934.—Further studies upon chemical factors affecting the breeding of Anophelines in Trinidad. *Bull. Ent. Res.*, 25 : 491.

DUNN, L. H., 1927.—Mosquito breeding in "test" water containers. *Bull. Ent. Res.*, 18 : 17.

HOPKINS, G. H. E., 1936.—Mosquitoes of the Ethiopian region. Part I.

LEE, D. J., 1944.—An atlas of the mosquito larvae of the Australasian region.

MACGREGOR, M. E., 1929.—The significance of pH in the development of mosquito larvae. *Parasitology*, 21 : 132.

SENIOR-WHITE, R., 1934.—Three years' mosquito work in Calcutta. *Bull. Ent. Res.*, 25 : 551.

THOMSON, R. C. M., 1940.—Studies on the behaviour of *Anopheles minimus*. Parts I, II and III. *J. Malar. Inst. Ind.*, 3 : 265, 295 and 323.

————, 1941.—*Id.*, Part IV. The composition of water and the influence of organic pollution on the selection of breeding place. *Ibid.*, 4 : 63.

WOODHILL, A. R., 1936.—Observations and experiments on *Aëdes concolor* Tayl. *Bull. Ent. Res.*, 27 : 633.

————, 1941*a*.—The oviposition responses of three species of mosquitoes (*Aëdes aegypti*, *Aëdes concolor* and *Culex fatigans*) in relation to the salinity of the water. PROC. LINN. SOC. N.S.W., 66 : 287.